UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

March 31, 2009

MEMORANDUM

Subject: Efficacy Review for PERACLEAN 15, EPA Reg. No 54289-4; DP Barcode: D360678.

From: Ibrahim Laniyan, Microbiologist
Product Science Branch
Antimicrobials Division (7510P)

Thru: Tajah Blackburn, Team Leader
Product Science Branch
Antimicrobials Division (7510P)

4/1/09

Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510P)

To: Marshall Swindell / Karen Leavy
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: Evonik Degussa Corporation
379 Interpace Parkway
Parsippany, NJ 07054

Formulation from the Label:

Active Ingredient(s)	% by wt.
Hydrogen Peroxide	22.0 %
Peroxyacetic Acid	15.0 %
Inert Ingredients	63.0 %
Total	100.0 %

I. BACKGROUND

The product, PERACLEAN 15 (EPA Reg. No. 54289-4), is an EPA-approved disinfectant (bactericide, fungicide), sanitizer, and deodorizer for use on hard, non-porous surfaces in industrial, institutional, commercial, animal care, and hospital or medical environments. The label claims that the product is effective as a disinfectant in the presence of 400 ppm hard water (as $CaCO_3$) and 5% fetal bovine serum. The applicant requested to amend the registration of this product to add claims for effectiveness as a disinfectant – when used with the 3M™ Twist 'n Fill™ System – against numerous bacteria, fungi, and viruses. According to the proposed label, the 3M™ Twist 'n Fill™ System will dilute PERACLEAN 15 with the appropriate amount of water to achieve 1500 ppm hydrogen peroxide and 1000 ppm peracetic acid. Studies were conducted at MicroBioTest, Inc., located at 105 Carpenter Drive, in Sterling, VA 20164.

This data package contained a letter from the applicant to EPA (dated November 7, 2008), thirty eight studies (MRID 476308-01 through -20, 476119-13, 476119-18 through 25, and 476119-27 through -35), Statements of No Data Confidentiality Claims for all thirty eight studies, and the proposed label.

Note: The laboratory reports describe studies conducted for the product, Experimental Liquid Cleaning Solution. The data package does not contain a statement confirming that the tested product, Experimental Liquid Cleaning Solution, is the product, PERACLEAN 15, which is the subject of this efficacy report. The laboratory reports do state that the diluted product contains 1500 ppm hydrogen peroxide and 1000 ppm peracetic acid, which are the same active ingredient concentrations identified on the proposed label for PERACLEAN 15 when used with the 3M™ Twist 'n Fill™ System.

II. USE DIRECTIONS

The product is designed for disinfecting hard, non-porous surfaces such as bathroom fixtures, bed frames, carts, chairs, coolers, countertops, doors, floors, racks, refrigerators, shelves, sinks, tables, and walls. The proposed label indicates that the product may be used on hard, non-porous surfaces including: aluminum, baked enamel, ceramic, chrome, glass, glazed porcelain, laminated surfaces, linoleum, painted surfaces, plastic (e.g., polypropylene, polyethylene), sealed stone, stainless steel, tile, and vinyl. Directions on the proposed label provide the following information regarding use of the product as a disinfectant when used with the 3M™ Twist 'n Fill™ System which provides a **0.853 ounce per gallon** dilution of the product: Apply use solution with a cloth, mop, sponge, auto-scrubber, or hand-pumped trigger sprayer such that all surfaces remain wet for 1 minute (3 minutes for *Clostridium perfringens* [sic; should be *Streptococcus pneumoniae*] and Vancomycin-Resistant *Enterococcus faecalis*; and 10 minutes for Poliovirus type 1 and *Mycobacterium bovis*). Allow surface to air dry. For heavily soiled areas, a preliminary cleaning is recommended.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments: The effectiveness of disinfectants for use on hard surfaces in hospital or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products as Disinfectants Method

(for spray products). Sixty carriers must be tested with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old, against *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708; formerly *Salmonella choleraesuis*), and *Pseudomonas aeruginosa* (ATCC 15442). To support products labeled as "disinfectants," killing on 59 out of 60 carriers is required to provide effectiveness at the 95% confidence level.

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria): The effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required. In addition, plate count data must be submitted for each microorganism to demonstrate that a concentration of at least 10^4 microorganisms survived the carrier-drying step.

Disinfectants for Use as Tuberculocides (Using the AOAC Tuberculocidal Activity of Disinfectants Test Method with modifications): Disinfectants may bear additional label claims of effectiveness as tuberculocides when supported by appropriate tuberculocidal effectiveness data. Certain chemical classes (i.e., glutaraldehyde and quaternary ammonium compounds) are required to undergo validation testing in addition to basic testing. Products that are formulated with other chemical groups do not require validation testing. When using the existing or modified AOAC Tuberculocidal Activity Test Methods, 10 carriers for each of 2 samples, representing 2 different product lots, must be tested against *Mycobacterium bovis* BCG (a member of the *Mycobacterium tuberculosis* species complex). Killing on all carriers/slides as demonstrated in Modified Proskauer-Beck Broth, and no growth in any of the inoculated tubes of 2 additional media (i.e., Middlebrook 7H9 Broth Difco B, Kirchners Medium, and/or TB Broth Base) is required.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi, Using a Modified AOAC Use-Dilution Method): The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The AOAC Use-Dilution Method may be modified to conform with the appropriate elements in the AOAC Fungicidal Test. The inoculum in the test must be modified to provide a concentration of at least 10^6 conidia per carrier. Ten carriers on each of 2 product samples representing 2 different product lots must be employed in the test. Killing of the specific pathogenic fungi on all carriers is required.

Note: As an interim policy, EPA is accepting studies with dried carrier counts that are at least 10^4 for *Trichophyton mentagrophytes*, *Aspergillus niger*, and *Candida albicans*. EPA recognizes laboratories are experiencing problems in maintaining dried carrier counts at the 10^6 level. This interim policy will be in effect until EPA determines that the laboratories are able to achieve consistent carrier counts at the 10^6 level.

Virucides: The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the

specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Supplemental Claims: An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum. On a product label, the hard water tolerance level may differ with the level of antimicrobial activity (e.g., sanitizer vs. disinfectant) claimed. To establish efficacy in hard water, all microorganisms (i.e., bacteria, fungi, viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same hard water tolerance level.

IV. COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 476308-01 "AOAC Use Dilution Test Supplemental Using *Serratia marcescens*" for Experimental Liquid Cleaning Solution, by Felicia L. Sellers. Study conducted at MicroBioTest, Inc. Study completion date – July 28, 2008. Laboratory Project Identification Number 109-164.

This study was conducted against *Serratia marcescens* (ATCC 13880). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exception: the culture was incubated at 26±2°C (which differs from the AOAC method specification of 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute at 22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC method specifications of 48±2 hours at 36±1°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

2. MRID 476308-02 "AOAC Use Dilution Test Supplemental Using *Salmonella enterica* serovar Typhimurium" for Experimental Liquid Cleaning Solution, by Kathryn D. Dormstetter. Study conducted at MicroBioTest, Inc. Study completion date – July 25, 2008. Laboratory Project Identification Number 109-163.

This study was conducted against *Salmonella enterica* serovar Typhimurium (ATCC 13311). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exceptions: the culture was incubated for 47.5 hours at 37±2°C (which differs from the AOAC method specifications of 48-54 hours at 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 47.5 hour old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute at 22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC method specifications of 48±2 hours at 36±1°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

3. MRID 476308-03 "AOAC Use Dilution Test Supplemental Using *Listeria monocytogenes*" for Experimental Liquid Cleaning Solution, by Felicia L. Sellers. Study conducted at MicroBioTest, Inc. Study completion date – July 28, 2008. Laboratory Project Identification Number 109-162.

This study was conducted against *Listeria monocytogenes* (ATCC 19111). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods,

with the following exception: the culture was incubated at 37±2°C (which differs from the AOAC method specification of 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute at 22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC method specifications of 48±2 hours at 36±1°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

4. MRID 476308-04 "AOAC Use Dilution Test Supplemental Using *Klebsiella pneumoniae*" for Experimental Liquid Cleaning Solution, by Felicia L. Sellers. Study conducted at MicroBioTest, Inc. Study completion date – July 28, 2008. Laboratory Project Identification Number 109-161.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exception: the culture was incubated at 37±2°C (which differs from the AOAC method specification of 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute at 22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC method specifications of 48±2 hours at 36±1°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

5. MRID 476308-05 "AOAC Use Dilution Test Supplemental Using *Staphylococcus aureus* with reduced susceptibility to Vancomycin" for Experimental Liquid Cleaning Solution, by Kathryn D. Dormstetter. Study conducted at MicroBioTest, Inc. Study completion date – July 25, 2008. Laboratory Project Identification Number 109-168.

This study was conducted against *Staphylococcus aureus* with reduced susceptibility to Vancomycin (ATCC 700787). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exception: the culture was incubated at 37±2°C (which differs from the AOAC method specification of 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute at 22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC method specifications of 48±2 hours at 36±1°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, confirmation of the challenge microorganism, and antibiotic resistance.

Note: Antibiotic resistance of *Staphylococcus aureus* with reduced susceptibility to Vancomycin (ATCC 700787) was verified on a representative culture. Dilutions of the vancomycin antibiotic (i.e., 48, 24, 12, 6, 3, 1.5, and 0.75 ug/mL) were prepared using sterile deionized water. A prepared culture of the challenge microorganism was diluted 1:100 using Mueller Hinton Broth. Aliquots (1 mL each) of the diluted culture were inoculated into tubes containing the dilutions of the vancomycin antibiotic. All tubes were incubated for 24±2 hours and observed for growth. Results confirmed reduced susceptibility of the challenge microorganism to vancomycin. See pages 9, 12, and 13 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

6. MRID 476308-06 "AOAC Use Dilution Test Supplemental Using Community-Associated Methicillin-Resistant *Staphylococcus aureus*" for Experimental Liquid Cleaning Solution, by Kathryn D. Dormstetter. Study conducted at MicroBioTest, Inc. Study completion date – July 25, 2008. Laboratory Project Identification Number 109-167.

This study was conducted against Community-Associated Methicillin Resistant *Staphylococcus aureus* Genotype 300 (CI 08001; received from the University of Louisville Hospital). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exception: the culture was incubated at 37±2°C (which differs from the AOAC method specification of 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute at 22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC method specifications of 48±2 hours at 36±1°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, confirmation of the challenge microorganism, and antibiotic resistance.

Note: Antibiotic resistance of Community-Associated Methicillin Resistant *Staphylococcus aureus* Genotype 300 was verified on a representative culture. An individual Mueller Hinton Agar was streaked with the prepared culture in a crosshatch pattern. After crosshatching, an antibiotic disk was added to the center of the plate. The plate was incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of Community-Associated Methicillin Resistant *Staphylococcus aureus* Genotype 300 to oxacillin. See pages 8 and 19 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

7. MRID 476308-07 "AOAC Use Dilution Test Supplemental Using Methicillin-Resistant *Staphylococcus aureus*" for Experimental Liquid Cleaning Solution, by Kathryn D. Dormstetter. Study conducted at MicroBioTest, Inc. Study completion date – July 25, 2008. Laboratory Project Identification Number 109-166.

This study was conducted against Methicillin Resistant *Staphylococcus aureus* (ATCC 33592). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exception: the culture was incubated at 37±2°C (which differs from the AOAC method specification of 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute at 22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC method specifications of 48±2 hours at 36±1°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, confirmation of the challenge microorganism, and antibiotic resistance.

Note: Antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* (ATCC 33592) was verified on a representative culture. An individual Mueller Hinton Agar was streaked with the prepared culture in a crosshatch pattern. After crosshatching, an antibiotic disk was added to the center of the plate. The plate was incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of Methicillin Resistant *Staphylococcus aureus* (ATCC 33592) to oxacillin. See pages 8, 18, and 19 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

8. MRID 476308-08 "AOAC Use Dilution Test Supplemental Using *Shigella dysenteriae* serotype 1" for Experimental Liquid Cleaning Solution, by Kathryn D. Dormstetter. Study conducted at MicroBioTest, Inc. Study completion date – July 25, 2008. Laboratory Project Identification Number 109-165.

This study was conducted against *Shigella dysenteriae* serotype 1 (ATCC 29026). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exception: the culture was incubated at 37±2°C (which differs from the AOAC method specification of 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder

carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute at 22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC method specifications of 48±2 hours at 36±1°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

9. MRID 476308-09 "AOAC Use Dilution Test Supplemental Using *Yersinia enterocolitica*" for Experimental Liquid Cleaning Solution, by Felicia L. Sellers. Study conducted at MicroBioTest, Inc. Study completion date – July 31, 2008. Laboratory Project Identification Number 109-172.

This study was conducted against *Yersinia enterocolitica* (ATCC 35669). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exception: the culture was incubated at 37±2°C (which differs from the AOAC method specification of 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute at 22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC method specifications of 48±2 hours at 36±1°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

10. MRID 476308-10 "AOAC Use Dilution Test Supplemental Using *Vibrio cholerae*" for Experimental Liquid Cleaning Solution, by Felicia L. Sellers. Study conducted at MicroBioTest, Inc. Study completion date – July 31, 2008. Laboratory Project Identification Number 109-176.

This study was conducted against *Vibrio cholerae* (ATCC 14035). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exception: the culture was incubated at 37±2°C (which differs from the AOAC method specification of 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute at 22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC method specifications of 48±2 hours at 36±1°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

11. MRID 476308-11 "AOAC Use Dilution Test Supplemental Using Methicillin-Resistant *Staphylococcus epidermidis*" for Experimental Liquid Cleaning Solution, by Kathryn D. Dormstetter. Study conducted at MicroBioTest, Inc. Study completion date – July 25, 2008. Laboratory Project Identification Number 109-169.

This study was conducted against Methicillin Resistant *Staphylococcus epidermidis* (ATCC 51625). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exception: the culture was incubated at 37±2°C (which differs from the AOAC method specification of 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder

carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute at 22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC method specifications of 48±2 hours at 36±1°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, confirmation of the challenge microorganism, and antibiotic resistance.

Note: Antibiotic resistance of Methicillin Resistant *Staphylococcus epidermidis* (ATCC 51625) was verified on a representative culture. An individual Mueller Hinton Agar was streaked with the prepared culture in a crosshatch pattern. After crosshatching, an antibiotic disk was added to the center of the plate. The plate was incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of Methicillin Resistant *Staphylococcus epidermidis* (ATCC 51625) to oxacillin. See pages 8, 18, and 19 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

12. MRID 476308-12 "AOAC Use Dilution Test Supplemental Using *Streptococcus pyogenes*" for Experimental Liquid Cleaning Solution, by Kathryn D. Dormstetter. Study conducted at MicroBioTest, Inc. Study completion date – July 25, 2008. Laboratory Project Identification Number 109-170.

This study was conducted against *Streptococcus pyogenes* (ATCC 19615). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exception: the culture was incubated at 37±2°C (which differs from the AOAC method specification of 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute at 22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 48±2 hours at 37±2°C under candle jar conditions (which differs from the AOAC method specifications of 48±2 hours at 36±1°C).

Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

13. MRID 476308-13 "AOAC Use Dilution Test Supplemental Using *Escherichia coli* O157:H7" for Experimental Liquid Cleaning Solution, by Felicia L. Sellers. Study conducted at MicroBioTest, Inc. Study completion date – July 28, 2008. Laboratory Project Identification Number 109-160.

This study was conducted against *Escherichia coli* O157:H7 (ATCC 35150). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exception: the culture was incubated at 37±2°C (which differs from the AOAC method specification of 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute at 22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC method specifications of 48±2 hours at 36±1°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

14. MRID 476308-14 "AOAC Use Dilution Test Supplemental Using Vancomycin-Resistant *Enterococcus faecalis*" for Experimental Liquid Cleaning Solution, by Kathryn D. Dormstetter. Study conducted at MicroBioTest, Inc. Study completion date – September 2, 2008. Laboratory Project Identification Number 109-159.

This study was conducted against Vancomycin-Resistant *Enterococcus faecalis* (ATCC 51575). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid

Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Initial testing was conducted using a 1-minute contact time. Repeat testing was conducted using a 3-minute contact time. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exceptions: the culture used during repeat testing was incubated for 47 hours (which differs from the AOAC method specification of 48-54 hours) and the cultures used during initial and repeat testing were incubated at 37±2°C (which differs from the AOAC method specification of 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour (or 47 hour) old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute (or 3 minutes) at 22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC method specifications of 48±2 hours at 36±1°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis (for repeat testing), confirmation of the challenge microorganism, and antibiotic resistance.

Note: Antibiotic resistance of Vancomycin-Resistant *Enterococcus faecalis* (ATCC 51575) was verified on a representative culture. An individual Mueller Hinton Agar was streaked with the prepared culture in a crosshatch pattern. After crosshatching, an antibiotic disk was added to the center of the plate. The plate was incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of Vancomycin-Resistant *Enterococcus faecalis* (ATCC 51575) to vancomycin. See page 8, 18, and 19 of the laboratory report.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

15. MRID 476308-15 "AOAC Use Dilution Test Supplemental Using *Clostridium perfringens*" for Experimental Liquid Cleaning Solution, by Kathryn D. Dormstetter. Study conducted at MicroBioTest, Inc. Study completion date – September 2, 2008. Laboratory Project Identification Number 109-158.

This study was conducted against *Clostridium perfringens* (ATCC 13124). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exceptions: the culture was incubated for 72±2 hours at 37±2°C under

anaerobic conditions (which differs from the AOAC method specifications of 48-54 hours at 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 72±2 hour old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute at 22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 72±2 hours at 37±2°C (which differs from the AOAC method specifications of 48±2 hours at 36±1°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: The applicant reported a failed trial set up on July 21, 2008. In that trial, no growth was observed in the carrier counts. Thus, the test was invalid. Testing was repeated on August 5, 2008 with acceptable results.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

16. MRID 476308-16 "AOAC Use Dilution Test Supplemental Using *Acinetobacter baumannii*" for Experimental Liquid Cleaning Solution, by Kathryn D. Dormstetter. Study conducted at MicroBioTest, Inc. Study completion date – July 25, 2008. Laboratory Project Identification Number 109-157.

This study was conducted against *Acinetobacter baumannii* (ATCC 19606). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exception: the culture was incubated at 37±2°C (which differs from the AOAC method specification of 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute at 22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC method specifications of 48±2 hours at 36±1°C). Following incubation, the subcultures were examined for the presence or absence of visible

growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

17. MRID 476308-17 "AOAC Use Dilution Test Supplemental Using *Candida albicans*" for Experimental Liquid Cleaning Solution, by Felicia L. Sellers. Study conducted at MicroBioTest, Inc. Study completion date – July 31, 2008. Laboratory Project Identification Number 109-175.

This study was conducted against *Candida albicans* (ATCC 10231). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exceptions: the culture was incubated for 72±2 hours at 37±2°C (which differs from the AOAC method specifications of 48-54 hours at 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 72±2 hour old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute at 22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 72±2 hours at 37±2°C (which differs from the AOAC method specifications of 48±2 hours at 36±1°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis [sic], and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

18. MRID 476308-18 "AOAC Use Dilution Test Supplemental Using *Streptococcus pneumoniae*" for Experimental Liquid Cleaning Solution, by Kathryn D. Dormstetter. Study conducted at MicroBioTest, Inc. Study completion date – September 19, 2008. Laboratory Project Identification Number 109-171.

This study was conducted against *Streptococcus pneumoniae* (ATCC 6304). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the

AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Initial testing was conducted using a 1-minute contact time. Repeat testing was conducted using a 3-minute contact time. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exceptions: the culture used during initial testing was incubated for 47 hours (which differs from the AOAC method specification of 48-54 hours) and the cultures used during initial and repeat testing were incubated at 37±2°C (which differs from the AOAC method specification of 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour (or 47 hour) old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute (or 3 minutes) at 21-22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC method specifications of 48±2 hours at 36±1°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, bacteriostasis (for repeat testing), and confirmation of the challenge microorganism.

Note: The study met GLP requirements with the following exception: The defibrinated sheep's blood used for transfers on September 9, 2008 had expired on September 5, 2008.

Note: The applicant reported failed trials set up on July 7, 2008 and August 29, 2008. In these trials, a lack of growth in the controls was observed. Thus, the tests were invalid. Testing was repeated on August 5, 2008 with acceptable results.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

19. MRID 476308-19 "AOAC Use Dilution Test Supplemental Using *Trichophyton mentagrophytes*" for Experimental Liquid Cleaning Solution, by Felicia L. Sellers. Study conducted at MicroBioTest, Inc. Study completion date – October 1, 2008. Laboratory Project Identification Number 109-173.

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods. The culture suspension was adjusted to yield approximately 5.0 x 10^6 by dilution with saline solution, as specified in the AOAC method (for fungicides). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10)

stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 3-5 day old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 26±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute at 24°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for up to 10 days at 23-30°C (which differs from the AOAC method specifications (for fungicides) of after 10 days at 25-30°C). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, fungistasis, and confirmation of the challenge microorganism.

Note: The applicant reported a failed trial set up on July 19, 2008. In this trial, insufficient growth was observed in the controls. Thus, this study was invalid. Testing was repeated on August 15, 2008 with acceptable results.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to carrier drying and subculture incubation. These deviations appear to be acceptable.

20. MRID 476308-20 "AOAC Tuberculocidal Activity of Disinfectants" for Experimental Liquid Cleaning Solution, by Felicia L. Sellers. Study conducted at MicroBioTest, Inc. Study completion date – October 30, 2008. Laboratory Project Identification Number 109-142.

This study was conducted against *Mycobacterium bovis* BCG (obtained from Organon Teknika, Corporation). Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Confirmative in vitro Test for Determining Tuberculocidal Activity (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. A culture of the challenge microorganism was prepared in accordance with the published AOAC method, with the following exception: the culture was incubated at 37±2°C (which differs from the AOAC method specification of 37°C). Heat-inactivated horse serum was added to the inoculum to achieve a 5% organic soil load. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). Ten (10) porcelain penicylinder carriers per product lot were immersed for 15 minutes in a 21-25 day old suspension of the test organism (15-20 mL inoculum per 10 carriers). The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 30 minutes at 37°C). Each carrier was placed in 10 mL of the use solution for 10 minutes at 22°C. The tubes containing the use solution were swirled after addition of the carriers. Following exposure, the carriers were transferred to individual tubes of 10 mL of DE Neutralizing Broth (which differs from the AOAC method specification of using horse serum to neutralize). After at least 10 minutes in the neutralizer, the carriers were shaken and transferred to individual tubes containing 20 mL of Modified Proskauer-Beck Medium. From each tube of neutralizer, 2.0 mL were cultured to tubes containing 20 mL of Middlebrook 7H9 Broth and 2.0 mL were cultured to tubes containing 20 mL of Kirchner's Medium. Each subculture tube was shaken, as specified in the AOAC method. All tubes used

for secondary transfers were incubated for 60 days at 37±2°C (which differs from the AOAC method specification of 37°C). The tubes were incubated for an additional 30 days because no growth was observed after 60 days. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, and confirmation of the challenge microorganism.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, product neutralization, and subculture incubation. These deviations appear to be acceptable.

21. MRID 476119-13 "AOAC Use Dilution Test" for Experimental Liquid Cleaning Solution, by Kathryn D. Dormstetter. Study conducted at MicroBioTest, Inc. Study completion date – April 14, 2008. Laboratory Project Identification Number 109-135.

This study was conducted against *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442). Three lots (Lot Nos. 129PAT7004, 129PAU6128, and 129PAT7002) of the product, Experimental Liquid Cleaning Solution, were tested using the AOAC Use-Dilution Method (modified) as described in the AOAC Official Methods of Analysis, 16th Edition, 2006 [sic]. At least one of the product lots (Lot No. 129PAT7002) was at least 60 days old at the time of testing. Use solutions were prepared by adding 1 part of the product and 150 parts of 400 ppm ± 2.9% AOAC hard water (a 1:151 dilution). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods, with the following exception: the culture was incubated at 37±2°C (which differs from the AOAC method specification of 36±1°C). Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers per product lot were immersed for 15 minutes in a 48-54 hour old suspension of the test organism, at a ratio of 20 carriers per tube of 20 mL broth. The carriers were dried for 20-40 minutes at 37±2°C (which differs from the AOAC method specifications of 40±2 minutes at 36±1°C). Each carrier was placed in 10 mL of the use solution for 1 minute at 22°C. The tubes containing the use solution were swirled after addition of the carriers, as specified in the AOAC methods. Following exposure, individual carriers were transferred to DE Neutralizing Broth to neutralize. The tubes containing neutralizer were shaken thoroughly after addition of the carriers, as specified in the AOAC methods. All subcultures were incubated for 48±2 hours at 37±2°C (which differs from the AOAC method specifications of 48±2 hours at 36±1°C). Due to the opacity of the neutralizer with *Pseudomonas aeruginosa*, cultures were streaked onto Tryptic Soy Agar plates and incubated for 24±2 hours at 37±2°C. Following incubation, the subcultures (and streaks) were examined for the presence or absence of visible growth. Controls included those for carrier counts, sterility, viability, neutralizer effectiveness, and confirmation of the challenge microorganisms.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing deviated from AOAC method specifications with regard to culture preparation, carrier drying, and subculture incubation. These deviations appear to be acceptable.

22. MRID 476119-18 "Virucidal Efficacy Test Using Herpes Simplex Virus Type 2" for Experimental Liquid Cleaning Solution, by S. Steve Zhou. Study conducted at MicroBioTest Inc. Study completion date – July 21, 2008. Laboratory Project Identification Number 109-148.

This study was conducted against Herpes simplex virus type 2 (ATCC VR-734), using Vero cells (ATCC CCL-81) as the host system. Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested according to a MicroBioTest Protocol titled "Virucidal Efficacy Test Using Herpes Simplex Virus Type 2," dated May 30, 2008. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). Serum was added to the viral stock to achieve an organic load of at least 5%. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 21-22°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 0.5% sodium thiosulfate and 0.2% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using RPMI with 5% newborn calf serum. Vero cells in multi-well culture dishes were inoculated in octuplicate with selected dilutions. The cultures were incubated for 6-8 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, plate recovery count, neutralizer effectiveness/viral interference, and cytotoxicity. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

23. MRID 476119-19 "Virucidal Efficacy Test Using Human Coronavirus Strain 229E" for Experimental Liquid Cleaning Solution, by S. Steve Zhou. Study conducted at MicroBioTest Inc. Study completion date – July 10, 2008. Laboratory Project Identification Number 109-147.

This study was conducted against Human coronavirus (Strain 229E; ATCC VR-740), using MRC-5 cells (ATCC CCL-171) as the host system. Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested according to a MicroBioTest Protocol titled "Virucidal Efficacy Test Using Human Coronavirus strain 229E," dated May 30, 2008. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). Serum was added to the viral stock to achieve an organic load of at least 5%. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 22°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 0.5% sodium thiosulfate and 0.2% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using EMEM with 10% fetal bovine serum, 1% penicillin/streptomycin, and 1% L-glutamine. MRC-5 cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 5-7 days at 33±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer (no data reported), plate recovery count, neutralizer effectiveness/viral interference, and cytotoxicity. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

24. MRID 476119-20 "Virucidal Efficacy Test Using Human Immunodeficiency Virus Type 1" for Experimental Liquid Cleaning Solution, by S. Steve Zhou. Study

conducted at MicroBioTest Inc. Study completion date – July 21, 2008. Laboratory Project Identification Number 109-149.

This study was conducted against Human immunodeficiency virus type 1 (strain not specified; obtained from Zeptometrix Corporation), using C8166 cells (obtained from the University of Pennsylvania) as the host system. Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested according to a MicroBioTest Protocol titled "Virucidal Efficacy Test Using Human Immunodeficiency Virus Type 1," dated May 30, 2008. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). Serum was added to the viral stock to achieve an organic load of at least 5%. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 22°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 0.5% sodium thiosulfate and 0.2% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using RPMI with 5% fetal bovine serum. C8166 cells in multi-well culture dishes were inoculated in octuplicate with selected dilutions. The cultures were incubated for 9-12 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, plate recovery count, neutralizer effectiveness/viral interference, and cytotoxicity. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: The testing conditions on page 7 of the laboratory report identify RPMI 1640 with 10% fetal bovine serum as the cell culture medium. The Project Sheet identifies RPMI with 5% fetal bovine serum as the cell culture medium.

25. MRID 476119-21 "Virucidal Efficacy Test Using Human Influenza A virus" for Experimental Liquid Cleaning Solution, by S. Steve Zhou. Study conducted at MicroBioTest Inc. Study completion date – August 19, 2008. Laboratory Project Identification Number 109-153.

This study was conducted against Human influenza A virus (Strain A/Hong Kong/8/68; H3N2; obtained from SPAFAS), using MDCK cells (ATCC CCL-34) as the host system. Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested according to a MicroBioTest Protocol titled "Virucidal Efficacy Test Using Human Influenza A Virus," dated May 30, 2008. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). Serum was added to the viral stock to achieve an organic load of at least 5%. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 21°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 0.5% sodium thiosulfate and 0.2% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using Minimum Essential Medium with 2.5 µg/mL trypsin. MDCK cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 4-6 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell

viability/sterility, plate recovery count, neutralizer effectiveness/viral interference, and cytotoxicity. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

26. MRID 476119-22 "Virucidal Efficacy Test Using Human Rotavirus" for Experimental Liquid Cleaning Solution, by Lauren A. Blaszak. Study conducted at MicroBioTest Inc. Study completion date – April 15, 2008. Laboratory Project Identification Number 109-138.

This study was conducted against Human rotavirus (Strain WA; ATCC VR-2018), using MA-104 cells (obtained from Charles River Laboratories) as the host system. Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested according to a MicroBioTest Protocol titled "Virucidal Efficacy Test Using Human Rotavirus," dated March 7, 2008. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). Serum was added to the viral stock to achieve an organic load of at least 5%. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 23°C. Following exposure, the plates were neutralized with 2.0 mL of fetal bovine serum with 0.5% sodium thiosulfate and 1% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using RPMI with 5% fetal bovine serum. MA-104 cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 7-9 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, plate recovery count, neutralizer effectiveness/viral interference, and cytotoxicity. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: The testing conditions on page 7 of the laboratory report identify Minimum Essential Medium with 1 mg/mL trypsin as the cell culture medium. The Project Sheet identifies RPMI with 5% fetal bovine serum as the cell culture medium.

27. MRID 476119-23 "Virucidal Efficacy Test Using Poliovirus Type 1" for Experimental Liquid Cleaning Solution, by S. Steve Zhou. Study conducted at MicroBioTest Inc. Study completion date – May 23, 2008. Laboratory Project Identification Number 109-140.

This study was conducted against Poliovirus type 1 (ATCC VR-1562), using Vero cells (ATCC CCL-81) as the host system. Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested according to a MicroBioTest Protocol titled "Virucidal Efficacy Test Using Poliovirus Type 1," dated May 13, 2008. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). Serum was added to the viral stock to achieve an organic

load of at least 5%. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 10 minutes at 25°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 0.5% sodium thiosulfate and 0.2% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using RPMI with 5% newborn calf serum, 1% penicillin/streptomycin, and 1% L-glutamine. Vero cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 6-9 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, plate recovery count, neutralizer effectiveness/viral interference, and cytotoxicity. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

28. MRID 476119-24 "Virucidal Efficacy Test Using Respiratory Syncytial Virus" for Experimental Liquid Cleaning Solution, by S. Steve Zhou. Study conducted at MicroBioTest Inc. Study completion date – July 21, 2008. Laboratory Project Identification Number 109-151.

This study was conducted against Respiratory syncytial virus (ATCC VR-26), using Hela cells (obtained from Diagnostic Hybrids) as the host system. Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested according to a MicroBioTest Protocol titled "Virucidal Efficacy Test Using Respiratory Syncytial Virus," dated May 30, 2008. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). Serum was added to the viral stock to achieve an organic load of at least 5%. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 23°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 0.5% sodium thiosulfate and 0.2% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using DMEM with 5% fetal bovine serum, 1% penicillin/streptomycin, and 1% L-glutamine. Hela cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 3-6 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, plate recovery count, neutralizer effectiveness/viral interference, and cytotoxicity. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

29. MRID 476119-25 "Virucidal Efficacy Test Using Rhinovirus Type 37" for Experimental Liquid Cleaning Solution, by S. Steve Zhou. Study conducted at MicroBioTest Inc. Study completion date – July 31, 2008. Laboratory Project Identification Number 109-150.

This study was conducted against Rhinovirus type 37 (Strain 151-1; ATCC VR-1147), using H1-Hela cells (ATCC CRL-1958) as the host system. Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested according to a MicroBioTest Protocol titled "Virucidal Efficacy Test Using Rhinovirus Type 37," dated May 30, 2008. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). Serum was added to the viral stock to achieve an organic load of at least 5%. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 23°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 0.5% sodium thiosulfate and 0.2% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using RPMI 1640 with 5% fetal bovine serum, 1% penicillin/streptomycin, and 1% L-glutamine. H1-Hela cells in multi-well culture dishes were inoculated in octuplicate with selected dilutions. The cultures were incubated for 6-9 days at 33±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, plate recovery count, neutralizer effectiveness/viral interference, and cytotoxicity. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

30. MRID 476119-27 "Virucidal Efficacy Test Using Avian Influenza virus" for Experimental Liquid Cleaning Solution, by S. Steve Zhou. Study conducted at MicroBioTest Inc. Study completion date – July 21, 2008. Laboratory Project Identification Number 109-154.

This study was conducted against Avian influenza virus type A (Strain Turkey/Wis/66; H9N2; obtained from SPAFAS), using MDCK cells (ATCC CCL-34) as the host system. Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested according to a MicroBioTest Protocol titled "Virucidal Efficacy Test Using Avian Influenza virus," dated May 30, 2008. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). Serum was added to the viral stock to achieve an organic load of at least 5%. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 22°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 0.5% sodium thiosulfate and 0.2% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using Minimum Essential Medium with 1 µg/mL trypsin. MDCK cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 4-6 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, plate recovery count, neutralizer effectiveness/viral interference, and cytotoxicity. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

31. MRID 476119-28 "Initial Virucidal Efficacy Test, Bovine Viral Diarrhea virus (Surrogate for Human Hepatitis C virus)" for Experimental Liquid Cleaning Solution, by Lauren A. Blaszak. Study conducted at MicroBioTest Inc. Study completion date – July 31, 2008. Laboratory Project Identification Number 109-145.

This study, under the direction of Study Director Lauren A. Blaszak, was conducted against Bovine viral diarrhea virus (strain not specified; obtained from American BioResearch Laboratories), using MDBK cells (ATCC CCL-22) as the host system. Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested according to a MicroBioTest Protocol titled "Initial Virucidal Efficacy Test, Bovine Viral Diarrhea Virus (Surrogate for Human Hepatitis C virus)," dated May 30, 2008. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). Serum was added to the viral stock to achieve an organic load of at least 5%. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. Two replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 22-23°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 0.5% sodium thiosulfate and 0.2% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using Minimum Essential Medium with 5% horse serum. MDBK cells in multi-well culture dishes were inoculated in octuplicate with selected dilutions. The cultures were incubated for 5-7 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, plate recovery count, neutralizer effectiveness/viral interference, and cytotoxicity. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: The applicant reported two failed trials set up on June 19, 2008 and July 1, 2008. In both trials, the cell condition was poor and the cells detached from the plates. The cytopathic effects could not be determined. Thus, these studies were invalid. Testing was repeated on July 17, 2008 with acceptable results.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

32. MRID 476119-29 "Confirmatory Virucidal Efficacy Test, Bovine Viral Diarrhea virus (Surrogate for Human Hepatitis C virus)" for Experimental Liquid Cleaning Solution, by Tien V. Mai. Study conducted at MicroBioTest Inc. Study completion date – August 28, 2008. Laboratory Project Identification Number 109-146.

This confirmatory study, under the direction of Study Director Tien V. Mai, was conducted against Bovine viral diarrhea virus (strain not specified; obtained from American BioResearch Laboratories), using MDBK cells (ATCC CCL-22) as the host system. One lot (Lot No. 129PAT7004) of the product, Experimental Liquid Cleaning Solution, was tested according to a MicroBioTest Protocol titled "Confirmatory Virucidal Efficacy Test Bovine Viral Diarrhea

Virus (Surrogate for Human Hepatitis C virus)," dated May 30, 2008. The use solution was prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). Serum was added to the viral stock to achieve an organic load of at least 5%. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. Two replicates were tested. For the single product lot, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 22°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 0.5% sodium thiosulfate and 0.2% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using Minimum Essential Medium with 5% horse serum. MDBK cells in multi-well culture dishes were inoculated in octuplicate with selected dilutions. The cultures were incubated for 7-9 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, plate recovery count, neutralizer effectiveness/viral interference, and cytotoxicity. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

33. MRID 476119-30 "Virucidal Efficacy Test Using Vaccinia virus" for Experimental Liquid Cleaning Solution, by S. Steve Zhou. Study conducted at MicroBioTest Inc. Study completion date – July 31, 2008. Laboratory Project Identification Number 109-152.

This study was conducted against Vaccinia virus (ATCC VR-156), using Vero cells (ATCC CCL-81) as the host system. Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested according to a MicroBioTest Protocol titled "Virucidal Efficacy Test Using Vaccinia virus," dated May 30, 2008. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). Serum was added to the viral stock to achieve an organic load of at least 5%. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 22-23°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 0.5% sodium thiosulfate and 0.2% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using RPMI 1640 with 5% newborn calf serum. Vero cells in multi-well culture dishes were inoculated in octuplicate with selected dilutions. The cultures were incubated for 6-8 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, plate recovery count, neutralizer effectiveness/viral interference, and cytotoxicity. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: The study met GLP requirements with the following exception: The study was initiated without the study director's signature due to the study director's absence on the day of testing. This was an administrative error and was not found to have a significant impact on the quality and integrity of the study data. The study director reviewed and approved the protocol prior to testing and reviewed the data upon lab completion.

34. MRID 476119-31 "Initial Virucidal Efficacy Test, Duck Hepatitis B Virus (Surrogate for Human Hepatitis B virus)" for Experimental Liquid Cleaning Solution, by Tien V. Mai. Study conducted at MicroBioTest Inc. Study completion date – July 31, 2008. Laboratory Project Identification Number 109-155.

This study, under the direction of Study Director Tien V. Mai, was conducted against Duck hepatitis B virus (strain not specified; obtained from HepadnaVirus Testing), using primary duck hepatocytes (obtained from Metzer Farms) as the host system. Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested according to a MicroBioTest Protocol titled "Initial Virucidal Efficacy Test Duck Hepatitis B Virus (Surrogate for Human Hepatitis B virus)," dated May 30, 2008. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). The stock virus culture contained 100% duck serum as an organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. Two replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 23°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 0.5% sodium thiosulfate and 0.2% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using Leibovitz 15 Complete. Primary duck hepatocytes in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 20-30 hours at 36±2°C in 5% CO_2 for viral adsorption. Post-adsorption, the media was aspirated and the cultures were re-fed. The cultures were returned to incubation for 9-13 days at 36±2°C in 5% CO_2. The cultures were re-fed, as necessary. Following incubation, the infectious virus was assayed by an immunofluorescence assay. Controls included those for cell viability/sterility, plate recovery count, neutralizer effectiveness/viral interference, and cytotoxicity. The 50% fluorescent focus forming unit dose per mL ($FFFUD_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

35. MRID 476119-32 "Confirmatory Virucidal Efficacy Test, Duck Hepatitis B Virus (Surrogate for Human Hepatitis B virus)" for Experimental Liquid Cleaning Solution, by S. Steve Zhou. Study conducted at MicroBioTest Inc. Study completion date – September 2, 2008. Laboratory Project Identification Number 109-156.

This confirmatory study, under the direction of Study Director S. Steve Zhou, was conducted against Duck hepatitis B virus (strain not specified; obtained from HepadnaVirus Testing), using primary duck hepatocytes (obtained from Metzer Farms) as the host system. One lot (Lot No. 129PAT7004) of the product, Experimental Liquid Cleaning Solution, was tested according to a MicroBioTest Protocol titled "Confirmatory Virucidal Efficacy Test, Duck Hepatitis B Virus (Surrogate for Human Hepatitis B virus)," dated May 30, 2008. A use solution was prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). Serum was added to the viral stock to achieve an organic load of at least 5%. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. Two replicates were tested. For the single product lot, separate dried virus films

were exposed to 2.0 mL of the use solution for 1 minute at 22°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 0.5% sodium thiosulfate and 0.2% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using Leibovitz 15 Complete. Primary duck hepatocytes in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 20-30 hours at 36±2°C in 5±1% CO_2 for viral adsorption. Post-adsorption, the media was aspirated and the cultures were re-fed. The cultures were returned to incubation for 9-13 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the infectious virus was assayed by an immunofluorescence assay. Controls included those for cell viability/sterility, virus stock titer (no data reported), plate recovery count, neutralizer effectiveness/viral interference, and cytotoxicity. The 50% fluorescent focus forming unit dose per mL ($FFFUD_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

36. MRID 476119-33 "Initial Virucidal Efficacy Test Feline calicivirus (Surrogate for Human Norovirus)" for Experimental Liquid Cleaning Solution, by Lauren A. Blaszak. Study conducted at MicroBioTest Inc. Study completion date – July 21, 2008. Laboratory Project Identification Number 109-143.

This study, under the direction of Study Director Lauren A. Blaszak, was conducted against Feline calicivirus (strain not specified; obtained from the University of Ottawa), using CrFK cells (obtained from American BioResearch Laboratories) as the host system. Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested according to a MicroBioTest Protocol titled "Initial Virucidal Efficacy Test, Feline calicivirus (Surrogate for Human Norovirus)," dated May 30, 2008. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). Serum was added to the viral stock to achieve an organic load of at least 5%. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. Two replicates per product lot were tested. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 22°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 0.5% sodium thiosulfate and 0.2% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using RPMI 1640 with 5% newborn calf serum. CrFK cells in multi-well culture dishes were inoculated in octuplicate with selected dilutions. The cultures were incubated for 7-9 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, plate recovery count, neutralizer effectiveness/viral interference, and cytotoxicity. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

37. MRID 476119-34 "Confirmatory Virucidal Efficacy Test, Feline calicivirus (Surrogate for Human Norovirus)" for Experimental Liquid Cleaning Solution, by Tien V. Mai. Study conducted at MicroBioTest Inc. Study completion date – July 31, 2008. Laboratory Project Identification Number 109-144.

This confirmatory study, under the direction of Study Director Tien V. Mai, was conducted against Feline calicivirus (strain not specified; obtained from the University of

Ottawa), using CrFK cells (obtained from American BioResearch Laboratories) as the host system. One lot (Lot No. 129PAT7004) of the product, Experimental Liquid Cleaning Solution, was tested according to a MicroBioTest Protocol titled "Confirmatory Virucidal Efficacy Test Feline calicivirus (Surrogate for Human Norovirus)," dated May 30, 2008. A use solution was prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). Serum was added to the viral stock to achieve an organic load of at least 5%. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. Two replicates were tested. For the single product lot, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 24°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 0.5% sodium thiosulfate and 0.2% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using RPMI 1640 with 5% newborn calf serum, 1% penicillin/streptomycin, and 1% L-glutamine. CrFK cells in multi-well culture dishes were inoculated in octuplicate with selected dilutions. The cultures were incubated for 7-9 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, virus stock titer, plate recovery count, neutralizer effectiveness/viral interference, and cytotoxicity. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

38. MRID 476119-35 "Virucidal Efficacy Test Using Herpes Simplex Virus Type 1" for Experimental Liquid Cleaning Solution, by Lauren A. Blaszak. Study conducted at MicroBioTest Inc. Study completion date – March 31, 2008. Laboratory Project Identification Number 109-137.

This study was conducted against Herpes simplex virus type 1 (ATCC VR-260), using Vero cells (ATCC CCL-81) as the host system. Two lots (Lot Nos. 129PAT7004 and 129PAU6128) of the product, Experimental Liquid Cleaning Solution, were tested according to a MicroBioTest Protocol titled "Virucidal Efficacy Test Using Herpes Simplex Virus Type 1," dated March 7, 2008. Use solutions were prepared by adding 1 part of the product and 149 parts of 400 ppm ± 2.9% AOAC hard water (a 1:150 dilution). Serum was added to the viral stock to achieve an organic load of at least 5%. Films of virus were prepared by spreading 0.2 mL of virus inoculum over pre-marked undersides of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 1 minute at 23°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 0.5% sodium thiosulfate and 0.2% Catalase. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using RPMI 1640 with 5% newborn calf serum. Vero cells in multi-well culture dishes were inoculated in octuplicate with selected dilutions. The cultures were incubated for 6-8 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability/sterility, plate recovery count, neutralizer effectiveness/viral interference, and cytotoxicity. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.